THE LETHAL EFFECT OF PURE DISTILLED WATER ON THE VINEGAR EEL (ANGUILLULA ACETI).[1]

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The question of the toxicity of distilled water for aquatic organisms appears to be still an open one. An excellent summary of the literature of the subject has been given by Bullot.[2] Ringer first noted that distilled water is toxic for a variety of organisms, and attributed this toxicity to (1) an abstraction from the organism of salts which are necessary for its life, or (2) the penetration of water into the cells through osmosis, or (3) the imbibition of water by the "intercellular substance." About the same time a contrary opinion was advanced by Nägeli[3] who claimed that it is not the purity of the water but the presence of the slightest trace of copper (even so small a quantity as 1/77,000,000) that gives water its toxic qualities, whereas if water of this sort is redistilled in glass it loses its toxicity. Shortly after this, Locke questioned Ringer's conclusions and decided that the latter's results too were due to the presence of copper rather than the toxicity of the water and in 1895[4] confirmed his belief by experiments with the tadpole and *Tubifex*. Ringer[5] then retested his experiments and partially recalled his former conclusions, agreeing with Locke that "pure water (*i. e.*, that distilled in glass) is completely harmless for the animals in question. This was confirmed by Jennings for *Paramecium*, Miss A. Moore for trout and tadpole and F. R. Lillie for *Planaria*. On the other hand Lyon[6] investigated the action of distilled water on developing *Arbacia* larvæ and found that artificial sea water made up of tap water or ordinary distilled water, whether vaporized in copper or glass, is very toxic for these larvæ although tap water which has

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[2] Bullot, *Univ. of Calif. Pub., Physiol.*, I., 1904, 199.

[3] Nägeli, *Denksch. d. Schweiz. Naturforsch. Gesells.*, Bd. 33, 1893.

[4] Locke, *Jour. Phys.*, 18, 1895, 319.

[5] Ringer, *Jour. Phys.*, 22, 1897.

[6] Lyon, BIOL. BULL., 6, 1904, 198.

been boiled long enough to concentrate it one third is much less toxic than ordinary distilled water. For this and other reasons he concluded that the chief toxic agent in such waters is the ammonia which they hold in solution. In fact he found that the larvæ often developed better in ammonia-free artificial sea water than in normal sea water, a result that may be due, as he suggests, to the presence of ammonia in natural sea water.

About the same time Bullot[1] published a thorough investigation of the toxicity of distilled water for the fresh water *Gammarus* and found that the water distilled over glass with all the precaution employed in a physical chemistry laboratory was toxic for this form although to a less extent than water distilled over copper. He found, however, that the presence of NaCl in such a dilution as is expressed by a concentration of 0.00008*N* enables the *Gammarus* to live indefinitely in such a medium. Loeb[2] suggests that in such a case, "The presence of a trace of NaCl in the distilled water possibly preserves the membrane better or maintains better the secretory activity of the cells, so that the animal can be freed from the excess of water which diffuses into it."

The group of Nematodes is noted for the extraordinary resistance which the cuticle offers to external media. The common vinegar or paste eel, *Anguillula aceti*, is well known to occur normally in weak vinegar, although acetic acid is to most organisms a rapidly acting poison. *Anguillula aceti* has been observed in our laboratory to live more than 24 hours in Tellyesnicky's fixing fluid and three or four hours in Gilson's fluid. The cuticle of this worm might therefore be expected to be quite unaffected by such ions as ordinary distilled water may contain. It is of interest to note in this connection that Devaine[3] in his biological investigation of the vinegar eel observed that the worms live at most but eight days in distilled water. This effect might be due to the lack of the acetic acid which ordinarily forms a part of their normal environment or to the direct effect of the water itself. It was soon found that although the worms live best in

[1] Bullot, *l. c.*

[2] Loeb, J., "Dynamics of Living Matter," p. 50, 1906.

[3] *Compt. Rend.*, T. 61, 1865, p. 259.

an environment of weak vinegar, it is the sugar rather than the acetic acid which appears to be the essential element of the medium.

With the hope of analysing the relations of this organism to its environment, a number of lines of investigation have been taken up in connection with the reactions of the nematodes to various media and to different food substances. The present paper deals only with the effect produced on the worms by distilled water. The worms used in these experiments were found in rather stale weak vinegar of an acidity of 8/10 *N*. No difficulty was experienced in breeding them abundantly in the laboratory both in this medium and in cider. Two kinds of distilled water were employed. One of these was such as is used in the ordinary work of the laboratory. This was distilled in an automatic copper still and will be designated "A." The second water, which will be designated "B," was obtained by distilling the first kind over potassium dichromate and sulphuric acid, condensing in block tin out of contact with the air and redistilling over barium hydrate. The first six tenths of this distillate was rejected and the remainder gave an extremely pure sample, CO_2-free and showing no ammonia reaction (Nesler test) even after the lapse of twelve hours. This water when tested in a conductivity apparatus of the department of physical chemistry gave no measurable conductivity with a resistance of 20,000 ohms and may be considered to be practically free from electrolytes. It was kept in well steamed, hard glass bottles. In each experiment every precaution was taken to remove all traces of the medium in which the worms were living. The worms were washed 6 to 8 times with distilled water in a centrifuge, and in the cases where the distilled water "B" was used, two or three times more in this. They were then allowed to remain in the "B" water for about 24 hours to insure a thorough rinsing. Finally they were again washed in pure water previous to their insertion in the new medium. The bottles and test tubes used to contain the worms in the first two sets of experiments were of ordinary soft glass and in the latter ones, *Schott and Genossen*, Jena resistance glass was employed. These containers were thoroughly soaked in chromic acid, and previous to use were repeatedly

rinsed in distilled water. They were then steamed for a greater or less length of time under pressure of 150 pounds. An attempt was made to put as nearly as possible the same number of worms in each culture tube but owing to their minute size it was of course impossible to do more than approximate the number.

Contrary to expectations the worms lived much longer in the ordinary laboratory water "A," presumably full of copper and ammonia than in that distilled over potassium dichromate and free from CO_2, NH_3, copper and other ions. In "A" and soft glass tubes they lived 31 days. In "B" water and soft tubes they lived sixteen days and in "B" water and Jena insoluble tubes they lived but six days. These results would seem to indicate that the presence of the electrolytes in ordinary distilled water and of the ions dissolved from the glass prolonged the life of the worms. Because of the fact that the purer the water the greater its solvent power on glass, it is still to be determined whether this effect is due to the substances dissolved from the glass or to the copper and other ions in the distilled water itself. In the series with "B" water and Jena glass, in which the worms lived but six days, there were no impurities in the water and practically no solvents from the glass. So we are forced to conclude that the toxic character of the water itself was the cause of the death of the worms. This is borne out by observations on the behavior of the worms under such conditions. The outer integument of the nematode in contrast to its normal hardness and resistance, appeared to become very viscous after a sojourn in pure water. When any obstacle was encountered in swimming, —the side of the tube or the surface of the water, or other worms, the organisms stuck fast. This alteration of the natural condition of the cuticle was so marked that after a time the worms bunched together in masses, unable to free themselves or else coming in contact with the surface of the water, they hung suspended. This effect would seem to bear out Loeb's suggestion quoted above. That there is a marked taking up of water on the part of the organism seems quite evident. The body becomes swollen and the worms lose their elasticity, becoming more or less rigid through turgor and swimming with the body almost in

a straight line without the characteristic twitching of the ends. The worms grow more and more impotent until at last instead of swimming directly towards the surface as is usually the case, they swim in a haphazard manner and in spite of instinctive efforts to reach the top of the water are unable to advance more than an inch or two, falling back towards the bottom with every effort. This may be due to decreased vitality but may also be due to a greater weight on account of the imbibition of water. Repetitions of these experiments have confirmed the results in every instance. Experiments have been undertaken to determine what ions control or effect the permeability of the cuticle to the water.

Summary.

1. *Anguillula aceti* succumbs in six days to very pure water free from electrolytes whereas in ordinary distilled water it will live for much longer periods.

2. The toxic qualities of the pure water appear to reside in its solvent effect on the normally resistant cuticle of the worm which is rendered much more permeable to water.

3. Death is accompanied by physical manifestations suggestive of great imbibition of water by the tissues.

4. The presence of electrolytes in the medium, derived either from solution of the glass culture tube or carried by "impure" samples of distilled water, prolongs the life of the organisms in question, apparently by preserving the character of the membrane so that the tissues will not imbibe water.